

**DATA EVALUATION RECORD  
FRESHWATER SEDIMENT *Chironomus riparius* EMERGENCE TEST  
(FOLLOWING OECD GUIDELINE 219)**

1. **CHEMICAL:** Ipconazole **PC Code:** 125618
2. **TEST MATERIAL:** Radiolabelled [benzylmethylene-<sup>14</sup>C] Ipconazole **Purity:** 98.1%

3. **CITATION:**

**Authors:** Flatman, D.  
**Title:** Ipconazole, Toxicity to the Sediment-Dwelling Phase of the Midge, *Chironomus riparius*.  
**Study Completion Date:** December 22, 2006  
**Laboratory:** Huntingdon Life Sciences, Ltd.  
Huntingdon, Cambridgeshire, England  
**Sponsor:** Kureha Corporation  
Chemicals and Agrochemicals Planning & Development Department  
Chuo-ku, Tokyo, Japan  
**Laboratory Report ID:** KRA/132  
**MRID No.:** 49910308  
**DP Barcode:** 434137

4. **REVIEWED BY:** Rebecca Bryan, Staff Scientist, CDM/CSS-Dynamac JV

**Signature:**  **Date:** 3/30/2017

**APPROVED BY:** Holly Rogers, Biologist, EPA/OPP/EFED/ERB5

**Signature:** **Date:** 05/04/2020

5. **APPROVED BY:** Hannah Yingling, Biologist, USEPA/OPP/EFED/ERB5

**Signature:**  **Date:** 04/28/2020

6. **DISCLAIMER:** This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel.

## 7. STUDY PARAMETERS

Scientific Name of Test Organism: *Chironomus riparius*  
Age of Test Organism: 1<sup>st</sup> instar larvae, <36 hours old  
Definitive Test Duration: 28 days  
Study Method: Static with aeration  
Type of Concentrations: Nominal, actual applied, and TWA for overlying water

## 8. CONCLUSIONS:

The chironomid *Chironomus riparius* was exposed to **Ipconazole** for 28 days in the spiked overlying water test. Chironomids were exposed to formulated sediment and overlying water dilution water that was spiked with ipconazole. Emergence of adult chironomids began on Day 13. Emergence rates, the sex ratio of emerging chironomids, and the development rate of chironomids to adults were assessed for contaminant effects. No mortality was observed during the test. Using time-weighted average concentrations in the overlying water, the NOAEC and LOAEC were calculated to be 0.077 and 0.17 mg <sup>14</sup>C-*Ipconazole*/L.

Assessment endpoints using time-weighted average concentrations in the overlying water:

NOAEC: 0.077 mg <sup>14</sup>C-*Ipconazole*/L

LOAEC: 0.17 mg <sup>14</sup>C-*Ipconazole*/L

Assessment endpoints: emergence rate, sex ratio in emergence, and development rate (sex-specific, combined)

Most sensitive endpoints: combined development rate, male development rate

## 9. ADEQUACY OF THE STUDY:

A. Classification: **This study is scientifically sound and is classified as acceptable.**

B. Rationale: This study followed methods described in OECD Guideline 219 (adopted 2004): “Sediment-Water Chironomid Toxicity Test Using Spiked Water” and does not fulfill any current U.S. EPA data requirement.

C. Reparability: N/A

## 10. MAJOR GUIDELINE DEVIATIONS (from OECD Guideline 219):

1. The TOC of the artificial sediment was not reported.

2. Physicochemical properties of ipconazole were not reported.

This deviation does not impact the acceptability of the study.

## 11. **MATERIALS AND METHODS**

**Stability of Compound Under Test Conditions:** For the overlying water samples from  $^{14}\text{C}$ -Ipconazole test solutions, the percent applied radioactivity of continuously decreased during the study from 90.5-94.6% on Day 0 to 49.5-55.7% on Day 1, and decreased to 8.3-9.9% by Day 28.

For the destructive samples at 0.219 and 3.52 mg  $^{14}\text{C}$ -Ipconazole/L, the percent applied radioactivity in overlying water decreased from 90.9 and 95.6% on Day 0 to 18.2 and 20.9% on Day 7, respectively. The pore water destructive samples were <0.1% applied radioactivity at Days 0 and 7. The sediment destructive samples increased from 7.46 and 7.36% on Day 0 to 70.1 and 59.4% on Day 7, respectively for the 0.219 and 3.52 mg  $^{14}\text{C}$ -Ipconazole/L treatments. The vessel rinse for the Day 7 destructive samples contained 1.2 and 1.4% applied radioactivity, respectively. The overall percent applied radioactivity for the destructive samples at 0.219 and 3.52 mg  $^{14}\text{C}$ -Ipconazole/L decreased from 98.4 and 103.0% on Day 0 to 89.5 and 81.7% on Day 7, respectively.

For all treatment levels at 28 days, the percent applied radioactivity in the test system was 8.3-9.9% in the overlying water, <0.1% in the pore water, 60.2-76.6% in the sediment. The total percent radioactivity in the test system was 69.6-84.9% for all treatment groups.

### **Physicochemical properties of Ipconazole.**

Parameter	Values	Comments
Water solubility at 20°C	Not reported	
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

*OECD requires water solubility, stability in water and light, pK<sub>a</sub>, P<sub>ow</sub>, and vapor pressure of the test compound.*

**A. Test Organisms/Acclimation**

Guideline Criteria	Reported Information
<b><u>Species</u></b> <i>Chironomus riparius</i>	<i>Chironomus riparius</i>
<b><u>Source</u></b>	Laboratory cultures (originally obtained from the University of Wales)
<b><u>Culture Conditions</u></b> A reproduction and oviposit chamber should consist of an adult area, sufficiently large to allow swarming (minimum 30 x 30 x 30 cm), and an oviposit area. Crystallizing dishes or larger containers with a thin layer of quartz sand (5 to 10 mm) or Kieselgur (thin layer to a few mm) spread over the bottom and containing suitable water to a depth of several cm are suitable as an oviposit area. Environmental conditions: temperature 20±2°C; 16:8 hours light:dark (intensity ca. 1000 lux); air humidity ca. 60%	The midges were cultured in Pyrex glass dishes (24 x 19 cm) containing an artificial substrate and filled to a depth of ca. 5.5 cm with Elendt M4 culture medium. The larval rearing vessels were held in a sufficiently large enclosure (minimum 30 x 30 x 30 cm) to allow swarming of the emerged adults to ensure that copulation occurs. At least two cultures, set up from different egg masses, were maintained at any one time. Cultures were aerated with compressed air through narrow glass tubes at approximately 1 cm above the substrate surface.  Cultures were maintained at 20 ± 2°C with a 16-hour light:8-hour dark photoperiod (intensity not reported).
<b><u>Egg Mass Acclimation Period</u></b> Four to five days before test initiation freshly laid egg masses should be taken from cultures and maintained separately in culture medium, temperature change should not exceed 2°C per day.	Egg masses were stored under the same conditions as the cultures until they hatched after approximately 3 days.
<b><u>Age of Test Larvae</u></b> First instar (1 to 4 days post-hatch with confirmation)	1 <sup>st</sup> instar, <36 hours post-hatch (confirmed with microscope)

Guideline Criteria	Reported Information
<b><u>Food</u></b> Green algae (e.g., <i>Scenedesmus subspicatus</i> , <i>Chlorella vulgaris</i> ) or flaked fish food as a ground powder, suspension, or filtrate	Chironomid cultures were fed daily with a suspension of TetraMin <sup>®</sup> fish food in dilution water (1 g:100 ml Elendt M4), at a rate equivalent to 0.5 mg/larva per day for Days 0-9 and 1 mg/larva per day for the remaining culture period.
<b><u>Health of parent culture stock</u></b> Were parent chironomids in good health during the culture period?	Not reported

**B. Test System**

Guideline Criteria	Reported Information
<p><b><u>Test Materials</u></b></p>	<p>Test item: Radiolabelled [benzylmethylene-<sup>14</sup>C] Ipconazole  IUPAC name: (1<i>RS</i>,2<i>SR</i>,5<i>RS</i>;1<i>RS</i>,2<i>SR</i>,5<i>SR</i>)-2-(4-chlorobenzyl)-5-isopropyl-1-(1<i>H</i>-1,2,4-triazol-1-ylmethyl)cyclopentanol  CAS name: 2-[(4-chlorophenyl)methyl]-5-(1-methylethyl)-1-(1<i>H</i>-1,2,4-triazol-1-ylmethyl)cyclopentanol  CAS No.: 125225-28-7  Description: White powder  Batch No.: 89010 (radiolabelled lot no. CFQ 13952)  Purity: 98.1%  Storage: room temperature in darkness</p> <p>Radiolabelled specific activity: 6.405 MBq/mg  Radiolabelled purity: Ethyl acetate hexane 98.8%; Tetrahydrofuran hexane 99.1%  Radiolabelled storage: ≤-15°C</p>
<p><b><u>Type of Test System</u></b></p> <p>Static (static-renewal or flow-through of overlying water is evaluated on a chemical-specific basis). Distilled or deionized water may be added to overlying water once daily as needed to maintain volume.</p>	<p>Static with aeration (no evaporative losses were observed during this study).</p>
<p><b><u>Test Water</u></b></p> <p>Soft reconstituted water or water from a natural source is preferred. Dechlorinated tap water may be used if the test organism will survive in it for the duration of the culturing and testing without showing signs of stress.</p>	<p>Reconstituted water (Elendt M4 medium) was prepared with reverse osmosis-deionized water and mineral salts and vitamins; a detailed chemical composition was provided.</p> <p><u>Medium characterization (Day 0):</u>  Hardness: 254-276 mg/L as CaCO<sub>3</sub>  Ammonia: 0.0-1.4 mg/L as NH<sub>3</sub>-N</p>

Guideline Criteria	Reported Information
<p><b><u>Test Sediment</u></b> Formulated (reconstituted, artificial, or synthetic) sediment is recommended. Content of sediment by dry weight: 5% peat (dry) (pH 5.5-6.0) or alpha-cellulose, 75% quartz sand (&gt;50% in size range of 50-200 microns), 20% kaolinite clay (kaolinite content ca. 30%), CaCO<sub>3</sub> 0.05-0.1%). Moisture content 30-50%, TOC 2% (±0.5%) and pH 6.5 - 7.5. Natural sediment can be used if it is fully characterized, unpolluted, and free of organisms that might compete with or consume chironomids. (If solvent other than water will be used, sand content of artificial sediment is adjusted accordingly.)</p>	<p>Formulated (artificial) sediment was prepared on a dry weight basis using the following components: 75% industrial sand (acid washed fine sand), 5% sphagnum peat, 20% kaolinite clay, <i>ca.</i> 1% calcium carbonate (to adjust the pH to 6.6).</p> <p>Sediment characteristics not reported.</p>
<p><b><u>Sediment Conditioning</u></b> <u>Artificial sediment:</u> 7 days in flowing dilution water prior to test initiation, chambers may be aerated</p>	<p>Test vessels (sediment:water) were prepared and acclimated to test conditions 7 days prior to test initiation.</p>
<p><b><u>Introduction of Test Organisms</u></b> Twenty-four hours prior to test initiation aeration of chambers is stopped and organisms are added to the chambers. Aeration should not resume for at least 24 hours. At test initiation, the test substance is spiked into the overlying water column.</p>	<p>After the seven days of acclimation, twenty chironomid larvae at the first instar stage were added to each test vessel. Aeration was started after approximately 24 hours,</p>
<p><b><u>Water Spiking</u></b></p>	<p>Suitable amounts of prepared stock solutions (in DMF) were applied into the overlying water column.</p> <p>Negative control and solvent control groups were included in the test.</p>

Guideline Criteria	Reported Information
<p><b><u>Solvents</u></b>            If used, minimal (i.e., <math>\leq 0.1</math> ml/l) and same concentration in all treatments. Suitable solvents are acetone, ethanol, methanol, ethylene glycol monoethyl ether, ethylene glycol dimethyl ether, dimethylformamide or triethylene glycol. (OECD guidelines also allows use of dispersants: Cremophor RH40, Tween 80, methycellulose 0.01%, and HCO-40)</p>	<p>Dimethylformamide (DMF), 0.1 mL/L overlying water</p>
<p><b><u>Water Temperature</u></b>  <math>20^{\circ}\text{C} \pm 2^{\circ}\text{C}</math> (Should not deviate between vessels by more than <math>1^{\circ}\text{C}</math>.)</p>	<p>20 to <math>21^{\circ}\text{C}</math></p>
<p><b><u>pH</u></b>  <u>Sediment</u>: <math>7.0 \pm 0.5</math>  <u>Interstitial Water</u>:  <u>Overlying Water</u>: 6.0 to 9.0            (Should not vary by more than 1 unit during test)</p>	<p><u>Sediment</u>: 6.6 (pre-test)  <u>Interstitial Water</u>: Not reported  <u>Overlying Water</u>: 6.2 to 8.1</p>
<p><b><u>TOC</u></b>  <u>Sediment</u>: <math>2 \pm 0.5\%</math>  <u>Overlying Water</u>: 2 mg/L</p>	<p><u>Sediment</u>: Not reported  <u>Overlying Water</u>: Not reported</p>
<p><b><u>Ammonia</u></b>  <u>Interstitial Water</u>:  <u>Overlying Water</u>:</p>	<p><u>Interstitial Water</u>: Not reported  <u>Overlying Water</u>: 0 to 1.4 mg/L on Day 0, 5.6 to 38.7 mg/L on Day 28</p>
<p><b><u>Total Water Hardness</u></b>            200 mg/L as <math>\text{CaCO}_3</math> (prefer 160 to 180 mg/L as <math>\text{CaCO}_3</math>)             (OECD 219 specifies that total hardness not exceed 400 mg/L as <math>\text{CaCO}_3</math>)</p>	<p>254 to 276 mg/L as <math>\text{CaCO}_3</math> on Day 0,            284 to 298 mg/L as <math>\text{CaCO}_3</math> on Day 28</p>
<p><b><u>Dissolved Oxygen</u></b>            60% air saturation value throughout test</p>	<p>7.4 to 8.9 mg <math>\text{O}_2</math>/L (<math>\geq 81\%</math> saturation)</p>



Guideline Criteria	Reported Information
<p><b><u>Aeration</u></b> Aeration (ca. one bubble/sec) is allowed except for when larvae are being added and for at least 24 hours after introduction of test organisms to a test chamber. If one test chamber is aerated all test chambers must be treated the same.</p>	<p>Aeration was provided using a narrow bore glass tube that was secured 2 - 3 cm above the sediment layer to avoid disturbance.</p> <p>Aeration was stopped during addition of test larvae and restarted 24 hours later.</p>
<p><b><u>Test Vessels or Compartments</u></b> 1. <u>Material</u>: Glass, No. 316 stainless steel, teflon or perfluorocarbon plastics 2. <u>Size</u>: Sediment depth of 1.5- 3 cm and the depth ratio of sediment to water should be ca. 1:4, must not be &gt;1:4; 600 ml beaker with 8 cm diameter</p>	<p>1. Glass beakers</p> <p>2. 8 cm diameter, containing a 2-cm depth of sediment (ca. 100 g) and a 400 mL of overlying water. The sediment to water depth ratio was 1:4.</p>
<p><b><u>Covers</u></b> Test vessels should be covered with a glass plate.</p>	<p>Test vessels were covered with plastic lids that were perforated to permit gaseous exchange.</p>
<p><b><u>Photoperiod</u></b> 16 hours light, 8 hours dark (Light intensity 500 to 1000 lux)</p>	<p>16-hour light/8-hour dark photoperiod (Light intensity not reported)</p>
<p><b><u>Food</u></b> Green algae (e.g., <i>Scenedesmus subspicatus</i>, <i>Chlorella vulgaris</i>) or flaked fish food as a ground powder, suspension, or filtrate</p>	<p>Aqueous suspension of TetraMin® fish food</p>
<p><b><u>Food Concentration and Frequency</u></b> Preferably feed daily but at least 3 times per week. <u>day 1 to 10</u>: 0.25-0.5 mg per larvae per day <u>remainder of test</u>: 0.5-1 mg per larvae per day (keep to a minimum, should not accumulate on sediment surface, cause overlying water to be cloudy or cause drop in DO)</p>	<p>Daily</p> <p><u>Day -1 to 10</u>: 0.5 mg/larva <u>Day 11 to test termination</u>: 1.0 mg/larva</p>

**C. Test Design**

Guideline Criteria	Reported Information
<b><u>Duration</u></b> <i>Chironomus riparius</i> : 28 days (if midges emerge early the test can be terminated after a minimum of 5 days after emergence of the last adult in the control).	28 days
<b><u>Nominal Concentrations</u></b> Negative control, solvent control (if a solvent was used) and at least 5 test concentrations. (Note exception to dilution factors described below can be made for shallow slope responses but minimum number of test concentrations may need to be increased)  <b><u>ECx endpoint</u></b> : test concentrations should bracket ECx and span the environmental concentration range. Dilution factor should not be greater than two between exposure concentrations.  <b><u>NOEC/LOEC endpoint</u></b> : factor between concentrations must not be greater than 3.	0 (negative control), 0 (DMF solvent control), 0.219, 0.438, 0.875, 1.75, and 3.5 mg ai/L  A nominal factor rate of 2 was used. The treatment levels were selected based on range-finding study (See Reviewer's Comments) and the EC <sub>50</sub> and NOAEC values were estimated.

Guideline Criteria	Reported Information
<p><b><u>Number of Test Organisms**</u></b></p> <p><u>ECx endpoint</u>: 60 larvae per treatment level; 3 replicates per treatment level</p> <p><u>NOAEC/LOAEC endpoint</u>: at least 80 larvae per treatment level with at least 4 replicates per treatment level (adequate power to detect a 20% difference, Type I error rate 5%)</p> <p>**(Optional) If data on 10-day growth and survival are needed additional replicates (number based on ECx or NOEC/LOEC endpoint determination) should be included at test initiation.</p>	<p><u>28-day endpoints</u>: 80 larvae per level: 20 larvae per replicate x 4 replicates for negative control and treated levels; x 8 replicates for solvent control (used for both EC<sub>50</sub> and NOAEC estimations).</p> <p>Four additional replicates (with chironomids) were prepared for analysis of overlying water, sediment, and pore water concentrations; two replicates at 0.219 mg/L and two replicates at 3.5 mg/L (referred to as “destructive sampling vessels”).</p> <p>**(Optional) 10-day growth data were not collected.</p>
<p><b>Test organisms randomly or impartially assigned to test vessels?</b></p>	<p>Yes</p>
<p><b><u>Overlying Water Parameter Measurements</u></b></p> <ol style="list-style-type: none"> <li>1. Dissolved oxygen should be measured daily in all test chambers.</li> <li>2. Temperature and pH should be measured in all test chambers at the start and end of the test and at least once a week during the test.</li> <li>3. Temperature should be monitored at least hourly throughout the test in one test chamber.</li> <li>4. Hardness and ammonia should be measured in the controls and one test chamber at the highest concentration at the start and end of the test.</li> </ol>	<ol style="list-style-type: none"> <li>1. DO was measured in all test vessels weekly (on Days -0, 7, 14, 21, and 28).</li> <li>2.-3. Temperature and pH was measured in all test vessels weekly (on Days -0, 7, 14, 21, and 28).</li> <li>4. Hardness and total ammonia were measured in unspecified vessels of the control and one replicate at highest treatment level (3.5 mg ai/L) on Days 0 and 28.</li> </ol>

Guideline Criteria	Reported Information
<p><b><u>Chemical Analysis-Overlying Water</u></b> At a minimum must be analyzed at test initiation (i.e., one hour after introduction of test substance into the test chamber) and at the end of the test in at least the highest concentration and one lower concentration.</p>	<p>Overlying water samples from all treated test vessels and all solvent control/control vessels were collected on Days 0 (following application), 1, 3, 7, 14 and 28. Using additional destructive samples at 0.219 and 3.5 mg/L, overlying water samples were measured at Days 0 and 7.</p> <p>Samples of overlying water were analyzed for <sup>14</sup>C-<i>Ipconazole</i> using LSC. The LOD was 0.001 mg ai/L.</p>
<p><b><u>Interstitial Water and Sediment Isolation Method</u></b> Centrifugation (e.g., 10,000 g and 4°C for 30 min) is recommended. If test substance is demonstrated not to adsorb to filters, filtration may be acceptable.</p>	<p>Following removal of pore water, sediment samples were handshaken with acetone for approximately 30 seconds and placed in sonic water bath for 15 minutes, and then samples were centrifuged for 15 minutes at 2500 rpm.</p>
<p><b><u>Chemical Analysis-Interstitial Water</u></b> At a minimum must be analyzed at the end of the test in at least the highest concentration and one lower concentration.</p>	<p>Using additional destructive samples at 0.219 and 3.5 mg/L, pore water samples were measured at Days 0 and 7. Pore water samples from all treated test vessels and all solvent control/control vessels were measured at Day 28.</p> <p>Samples of pore water were analyzed for <sup>14</sup>C-<i>Ipconazole</i> using LSC. The LOD was 0.001 mg ai/L.</p>

Guideline Criteria	Reported Information
<b><u>Chemical Analysis-Bulk Sediment</u></b> At a minimum must be analyzed at the end of the test in at least the highest concentration and one lower concentration.	Using additional destructive samples at 0.219 and 3.5 mg/L, sediment samples were measured at Days 0 and 7. Sediment samples from all treated test vessels and all solvent control/control vessels were measured at Day 28.  Samples of sediment were analyzed for <sup>14</sup> C-Ipconazole using LSC. The LOD was 0.001 mg ai/L.

## 12. REPORTED RESULTS

### A. General Results

Guideline Criteria	Reported Information
<b>Quality assurance and GLP compliance statements were included in the report?</b>	Yes. This study was conducted in compliance with the UK, OECD, and EC Commission GLP standards.
<b><u>Control Mortality</u></b> <30%	No mortality reported.
<b>Did chironomids emerge in controls between day 12 and 23?</b>	Chironomids emerged in controls between Days 13 and 24.
<b><u>Control Emergence</u></b> Mean emergence between 50-70%	Negative control – 93.4% emergence Solvent control – 95.5% emergence

Guideline Criteria	Reported Information
<b><u>Data Endpoints</u></b> <u>Emergence Test (28 day)</u> - Number alive - Time to emergence - Number of emerged male and female midges - Number of visible pupae that have failed to emerge - Number of egg masses deposited - Observations of other effects, abnormal behavior, or appearance or clinical signs (e.g., leaving sediment, unusual swimming)  <u>Growth and Survival (10-day) (Optional)</u> - Number alive - Instar level of surviving larvae - Dry weight (ash free) per test chamber of surviving larvae by instar level	<u>Emergence Test (28 day)</u> - Number alive (emerged) - Time to emergence - Number of emerged male and female midges - Observations of other effects for larvae - Development rate was calculated          <u>Growth and Survival (10-day)</u> N/A
<b>Raw data included?</b>	Yes, sufficient

### **Effects Data**

Toxicity Observations: The percent of emerged chironomids averaged 92.3, 94.3, 90.6, 91.9, and 83.6% for the actual applied 0.219, 0.436, 0.874, 1.76, and 3.52 mg ai/L treatment levels, respectively, compared to 93.4% in the negative control and 95.5% in the solvent control. No significant differences ( $p > 0.05$ ) in percent emergence or in sex ratio in emergence were indicated at any treatment level compared to the solvent control. The reported NOAEC and LOAEC values for emergence and sex ratio in emergence were 3.52 and  $>3.52$  mg ai/L, respectively.

Table 1. Summary of  $^{14}\text{C}$ -Ipconazole effects on *Chironomus riparius* emergence success, sex ratio of emerging chironomids, and development rate.

Actual Applied (Nominal) Concentration (mg ai/L)	Initial No.	Number Emerged <sup>a</sup>			Mean % Emergence	Mean Development Rate (1/(day-0.5)) <sup>b</sup>
		Total	Male	Female		
Negative control	80	73	35	38	93.4	0.0682
Solvent control	161	150	72	78	95.5	0.0655
0.219 (0.219)	80	72	41	31	92.3	0.0635
0.436 (0.438)	80	74	29	45	94.3	0.0649
0.874 (0.875)	80	70	27	43	90.6	0.0597
1.76 (1.75)	80	71	25	45	91.9	0.0613
3.52 (3.5)	80	66	37	29	83.6	0.0600

(a) Reviewer-calculated from emergence data provided in Table 1 on page 26, and Appendix 3 on page 34 of the study report.

(b) Mean Development Rate calculated using the following formula:  $\text{DR} = 1/(\text{day}-0.5)$ , where day = the day on which emergence was recorded.

Development rates averaged 0.0635, 0.0649, 0.0597, 0.0613, and 0.0600 days<sup>-1</sup> for the actual applied 0.219, 0.436, 0.874, 1.76, and 3.52 mg ai/L treatment levels, respectively, compared to 0.0682 days<sup>-1</sup> in the negative control and 0.0655 days<sup>-1</sup> in the solvent control. No significant differences ( $p > 0.05$ ) in development rates were indicated at any treatment level compared to the solvent control. The reported NOAEC and LOAEC values for development rate were 3.52 and  $>3.52$  mg ai/L, respectively.

## B. Statistical Results (From Study Report)

Emergence, sex ratio in emergence, and development rate data were statistically analyzed using SAS 8.2 (1999) statistical software and were reported in terms of actual applied concentrations. The F1 test for monotonicity was first applied, and the data followed a monotonic dose-response relationship. For emergence success and development rate, each treated group was compared with the solvent control using a two-sided Williams' test for monotonic trend

on the transformed data and using the error mean square from a one-way analysis of variance. For percentage male emergents (sex ratio), each treated group was then compared with the solvent control using a Dunnett's test. Negative and solvent control data were compared using a t-test. The EC<sub>50</sub> values for emergence and development rate was observed to be greater than the highest test concentration level.

Most sensitive endpoint(s): None

Endpoint	Methods	NOAEC mg ai/L	LOAEC mg ai/L
28-d Emergence	William's t-test	3.52	>3.52
28-d Development Rate	William's t-test	3.52	>3.52
10-d Survival (Optional)	---	---	---
10-d Growth (Optional)	---	---	---

### 13. VERIFICATION OF STATISTICAL RESULTS

The reviewer analyzed percent emergence, male:female ratio of emerging chironomids, and development rate (sex-specific, combined) using CETIS statistical software version 1.9.2.8 with database backend settings implemented by EFED on 10/20/2015. For the percent emergence analysis, the reviewer used values calculated from Appendix 3 of the study report; these values differed than the study author's mean percents emergence (see table below, see Reviewer's Comments).

Negative and solvent control data were compared using a two-sample t-test assuming equal variance. Male development rate in the solvent control was significantly lower than in the negative control ( $p = 0.03$ ). No other differences were detected and all subsequent analyses were conducted by comparing treatment data to the negative control only. Data were then tested for normality using the Shapiro-Wilk's test ( $\alpha = 0.01$ ) and for homogeneity of variance using Bartlett's test ( $\alpha = 0.01$ ). Male:female ratio in emergence failed to meet these assumptions and was therefore analyzed using the non-parametric Mann-Whitney U Two-Sample test; all other endpoints were analyzed using analysis of variance followed by Dunnett's and Williams tests. All analyses were conducted using the reviewer-calculated time-weighted average (TWA) overlying water concentrations.

#### **Re-calculated Percent Emergence<sup>(a)</sup>.**

Time-Weighted Average (Nominal) Concentration (mg ai/L) <sup>(b)</sup>	Initial No.	Total No. Emergenced <sup>(c)</sup>	Mean % Emergence
Negative control	80	73	91.2
Solvent control	161	150	93.7



Time-Weighted Average (Nominal) Concentration (mg ai/L) <sup>(b)</sup>	Initial No.	Total No. Emergenced <sup>(c)</sup>	Mean % Emergence
0.040 (0.219)	81 <sup>(d)</sup>	72	88.7
0.077 (0.438)	80	74	92.5
0.17 (0.875)	80	70	87.5
0.32 (1.75)	80	70	87.5
0.70 (3.5)	80	66	82.5

(a) Reviewer re-calculated percents emergence as they differ from the study author for unknown reasons.

(b) The concentrations are based on TWA overlying water concentrations.

(c) These totals were calculated from Appendix 3 on page 34 of the study report, which are consistent with the study author with the exception of the 1.75 mg ai/L (nominal) treatment total.

(d) According to Appendix 3 on page 34 of the study report, 21 chironomids emerged from replicate R2 in the 0.219 mg ai/L treatment; therefore, the initial number of chironomids was increased by 1 chironomid.

#### Summary of Statistical Methods used for NOAEC/LOAEC Analyses.

Endpoint	Solvent vs Dilution Control		NOAEC/LOAEC	
	Method	Diff <sup>(1)</sup> (%)	Method	Diff <sup>(2)</sup> (%)
28-d Emergence Rate	Equal variance t-test	-2.67	Dunnett/Williams	9.59
Male:Female Ratio in Emergence	Equal variance t-test	10.09	Mann-Whitney	-295
Combined Development Rate	Equal variance t-test	4.08	Williams	4.88
Male Development Rate	Equal variance t-test	5.08	Williams	2.50
Female Development Rate	Equal variance t-test	2.32	Dunnett/Williams	3.83

<sup>(1)</sup> Difference between the mean dilution water and solvent control responses.

<sup>(2)</sup> Difference between the dilution water and NOAEC concentration treatment.

Most sensitive endpoint: development rate (male, combined)

**Verification Statistical Endpoint Values<sup>(a)</sup>.**

Statistical Endpoint	28-day Emergence	Male:Female Ratio in Emergence	Combined Development Rate	Male Development Rate	Female Development Rate
NOAEC (mg ai/L)	0.70	0.70	0.077	0.077	0.32
LOAEC (mg ai/L)	>0.70	>0.70	0.17	0.17	0.70

(a) Results are based on TWA overlying water concentrations.

**14. REVIEWER'S COMMENTS:**

The reviewer's conclusions were based on the time-weighted average overlying water concentrations and are reported in the Conclusion section of this DER. The reviewer's mean values for emergence differ from those reported by the study author, though it is not clear why. It is possibly due to a data entry error on the part of the study author. However, overall trends were comparable and neither the reviewer nor the study author detected a significant difference in emergence at any treatment level.

The study was designed to fulfill OECD Guideline 219 *Sediment-Water Chironomid Toxicity Test Using Spiked Water* (2004). Although this study does not fulfill any current U.S. EPA guideline requirement, there were no significant deviations from OECD Guideline 219 that would affect the scientific soundness of this study, as all validity requirements as delineated in OECD 219 guidance (2004) for a chironomid emergence toxicity test were fulfilled.

An acute 48-hour preliminary range-finding study with larvae was conducted at nominal non-radiolabelled concentrations of 0.05, 0.10, 0.50, 1.0, and 5.0 mg/L. Significant acute toxicity was observed at the highest test concentration of 5.0 mg/L.

A preliminary trial using the radiolabelled test material was conducted to establish the highest attainable concentration under definitive test conditions, except for the absence of sediment. The nominal 3.5 mg/L sample recoveries were consistent and ranged from 77 to 81% at the time of application, 5 hours after application, and 2 days after application. Based on the recoveries, the maximum attainable concentration under definitive test conditions was 2.70 to 2.84 mg ipconazole/L. The majority of the test material remained in solution, and the highest nominal concentration considered acceptable for definitive testing was 3.5 mg/L. This concentration (3.5 mg ai/L) was used as the highest test concentration in the definitive test.

The light intensity and TOC values were not reported for the definitive study.

For comparison purposes, time-weighted average (TWA) concentrations of overlying water was reviewer-calculated. Results were reported in terms of TWA concentrations in the Conclusions section of the DER. TWA concentrations were calculated using the following equation:

$$C_{TWA} = \frac{\left(\frac{C_1 + C_0}{2}\right)(t_1 - t_0) + \left(\frac{C_2 + C_1}{2}\right)(t_2 - t_1) + \left(\frac{C_{n-1} + C_{n-2}}{2}\right)(t_{n-1} - t_{n-2}) + \left(\frac{C_n + C_{n-1}}{2}\right)(t_n - t_{n-1})}{t_n}$$

where:

$C_{TWA}$  is the time-weighted average concentration,

$C_j$  is the concentration measured at time interval  $j$  ( $j = 0, 1, 2, \dots, n$ )

$t_j$  is the number of hours (or days or weeks, units used just need to be consistent in the equation) of the test at time interval  $j$  (e.g.,  $t_0 = 0$  hours (test initiation),  $t_1 = 24$  hours,  $t_2 = 96$  hours).

The reviewer-calculated time-weighted average concentrations of the overlying water are 0.040, 0.077, 0.17, 0.32, and 0.70 mg ai/L for the nominal 0.219, 0.438, 0.875, 1.75, and 3.5 mg ai/L groups, respectively. Pore water and sediment samples were only collected and analyzed for all test vessels on Day 28; additional replicates of the 0.219 and 3.5 mg ai/L (nominal concentrations) treatments were destructively sampled on Days 0 and 7. The total amount of radioactivity was determined in all matrices at all treatment levels, though the amount of sediment in each test vessel, based on dry weight, was not reported. Therefore, the reviewer could not estimate concentrations in bulk sediment.

The definitive exposure phase for this study was conducted from October 28 to November 25, 2005.

## 15. REFERENCES:

None; other than standard guidelines or methodologies

# CETIS Analytical Report

Report Date: 30 Jun-17 18:45 (p 1 of 14)  
Test Code: 125618 49910308 | 18-9896-9629

## OPPTS 850.1760 Chronic Sediment Chironomus Huntingdon Life Sciences

<b>Analysis ID:</b> 20-4171-5538	<b>Endpoint:</b> Combined Development Rate	<b>CETIS Version:</b> CETISv1.9.2
<b>Analyzed:</b> 30 Jun-17 18:41	<b>Analysis:</b> Parametric-Two Sample	<b>Official Results:</b> Yes
<b>Batch ID:</b> 08-0420-8106	<b>Test Type:</b> Chronic Sediment Chironomus	<b>Analyst:</b>
<b>Start Date:</b> 28 Oct-05	<b>Protocol:</b> OPPTS 850.1760 Chronic Sediment Chiron	<b>Diluent:</b> Elendt M4 Medium
<b>Ending Date:</b>	<b>Species:</b> Chironomus dilutus	<b>Brine:</b> Not Applicable
<b>Duration:</b> n/a	<b>Source:</b> Lab In-House Culture	<b>Age:</b> <36h

Data Transform	Alt Hyp	Comparison Result	PMSD
Untransformed	C <> T	Solvent Blank passed combined development	5.84%

### Equal Variance t Two-Sample Test

Control	vs	Control II	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		Solvent Blank	1.5	2.23	0.004	10	CDF	0.1654	Non-Significant Effect

### ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1.908E-05	1.908E-05	1	2.24	0.1654	Non-Significant Effect
Error	8.522E-05	8.522E-06	10			
Total	0.0001043		11			

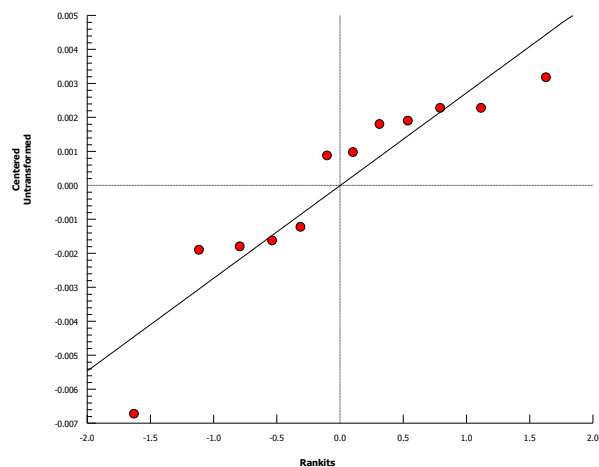
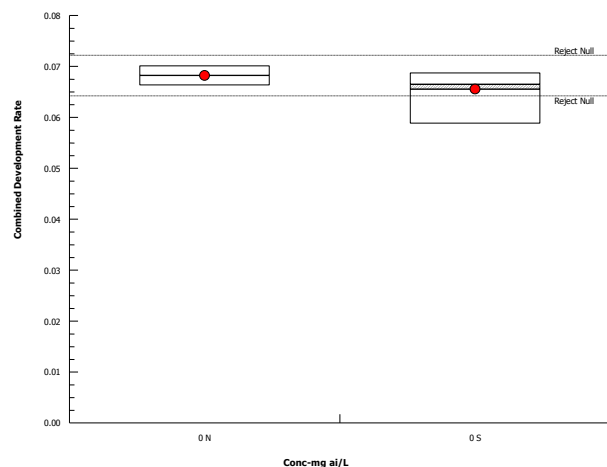
### Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F Test	2.24	44.4	0.5457	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.869	0.802	0.0639	Normal Distribution

### Combined Development Rate Summary

Conc-mg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	S	8	0.0655	0.0629	0.0682	0.0664	0.0588	0.0687	0.00113	4.88%	0.00%
0	N	4	0.0682	0.0648	0.0716	0.0682	0.0663	0.0701	0.00107	3.13%	-4.08%

### Graphics



# CETIS Analytical Report

Report Date: 30 Jun-17 18:45 (p 2 of 14)  
Test Code: 125618 49910308 | 18-9896-9629

## OPPTS 850.1760 Chronic Sediment Chironomus

Huntingdon Life Sciences

<b>Analysis ID:</b> 03-0201-9941	<b>Endpoint:</b> Combined Development Rate	<b>CETIS Version:</b> CETISv1.9.2
<b>Analyzed:</b> 30 Jun-17 18:42	<b>Analysis:</b> Parametric-Control vs Treatments	<b>Official Results:</b> Yes
<b>Batch ID:</b> 08-0420-8106	<b>Test Type:</b> Chronic Sediment Chironomus	<b>Analyst:</b>
<b>Start Date:</b> 28 Oct-05	<b>Protocol:</b> OPPTS 850.1760 Chronic Sediment Chiron	<b>Diluent:</b> Elendt M4 Medium
<b>Ending Date:</b>	<b>Species:</b> Chironomus dilutus	<b>Brine:</b> Not Applicable
<b>Duration:</b> n/a	<b>Source:</b> Lab In-House Culture	<b>Age:</b> <36h

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	0.7	> 0.7	n/a		12.37%

### Dunnett Multiple Comparison Test

Control	vs	Conc-mg ai/L	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		0.04	1.36	2.41	0.008	6	CDF	0.2774	Non-Significant Effect
		0.077	0.949	2.41	0.008	6	CDF	0.4447	Non-Significant Effect
		0.17	2.4	2.41	0.008	6	CDF	0.0503	Non-Significant Effect
		0.32	2.08	2.41	0.008	6	CDF	0.0903	Non-Significant Effect
		0.7	2.34	2.41	0.008	6	CDF	0.0567	Non-Significant Effect

### ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0002178	4.357E-05	5	1.77	0.1691	Non-Significant Effect
Error	0.0004421	2.456E-05	18			
Total	0.00066		23			

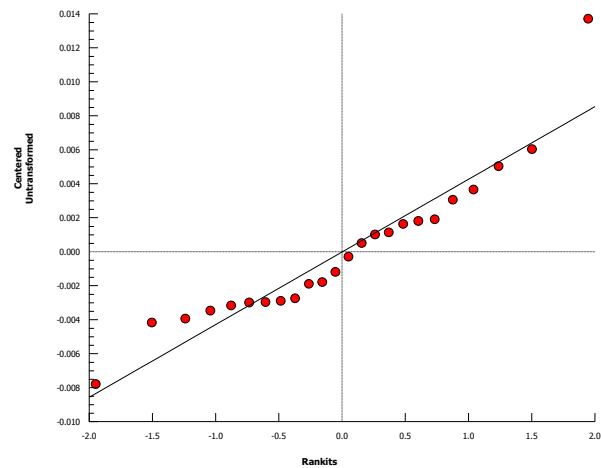
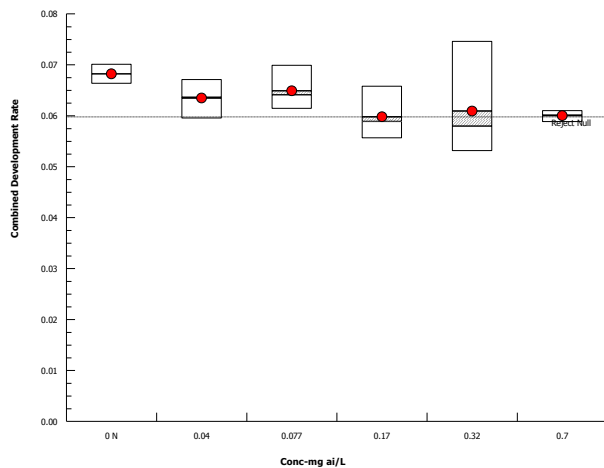
### Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance Test	12.4	15.1	0.0293	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.912	0.884	0.0384	Normal Distribution

### Combined Development Rate Summary

Conc-mg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	0.0682	0.0648	0.0716	0.0682	0.0663	0.0701	0.00107	3.13%	0.00%
0.04		4	0.0635	0.0572	0.0697	0.0636	0.0595	0.0671	0.00195	6.16%	6.96%
0.077		4	0.0649	0.0584	0.0714	0.0641	0.0614	0.0699	0.00204	6.30%	4.88%
0.17		4	0.0598	0.0524	0.0671	0.0588	0.0556	0.0658	0.00231	7.72%	12.35%
0.32		4	0.0609	0.0459	0.0759	0.0579	0.0531	0.0746	0.00471	15.46%	10.70%
0.7		4	0.06	0.0585	0.0615	0.0601	0.0588	0.061	0.000481	1.60%	12.02%

### Graphics



# CETIS Analytical Report

Report Date: 30 Jun-17 18:45 (p 3 of 14)  
Test Code: 125618 49910308 | 18-9896-9629

## OPPTS 850.1760 Chronic Sediment Chironomus

Huntingdon Life Sciences

<b>Analysis ID:</b> 18-5945-2666	<b>Endpoint:</b> Combined Development Rate	<b>CETIS Version:</b> CETISv1.9.2
<b>Analyzed:</b> 30 Jun-17 18:43	<b>Analysis:</b> Parametric-Control vs Ord.Treatments	<b>Official Results:</b> Yes
<b>Batch ID:</b> 08-0420-8106	<b>Test Type:</b> Chronic Sediment Chironomus	<b>Analyst:</b>
<b>Start Date:</b> 28 Oct-05	<b>Protocol:</b> OPPTS 850.1760 Chronic Sediment Chiron	<b>Diluent:</b> Elendt M4 Medium
<b>Ending Date:</b>	<b>Species:</b> Chironomus dilutus	<b>Brine:</b> Not Applicable
<b>Duration:</b> n/a	<b>Source:</b> Lab In-House Culture	<b>Age:</b> <36h

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	0.077	0.17	0.1144		9.59%

### Williams Multiple Comparison Test

Control	vs	Conc-mg ai/L	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		0.04	1.36	1.73	0.006	6	CDF	>0.05	Non-Significant Effect
		0.077	1.15	1.82	0.006	6	CDF	>0.05	Non-Significant Effect
		0.17*	2.4	1.85	0.006	6	CDF	<0.05	Significant Effect
		0.32*	2.24	1.86	0.007	6	CDF	<0.05	Significant Effect
		0.7*	2.34	1.87	0.007	6	CDF	<0.05	Significant Effect

### ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0002178	4.357E-05	5	1.77	0.1691	Non-Significant Effect
Error	0.0004421	2.456E-05	18			
Total	0.00066		23			

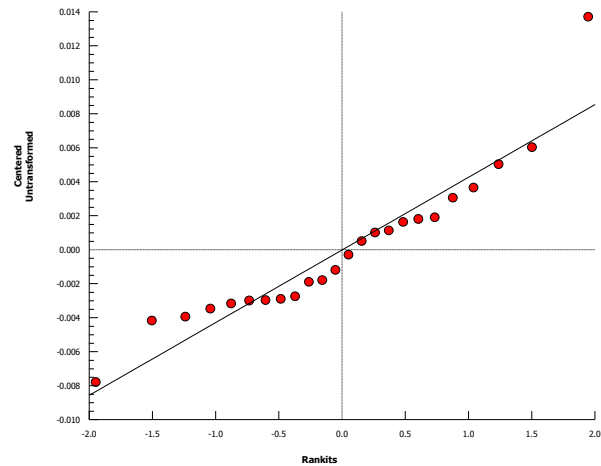
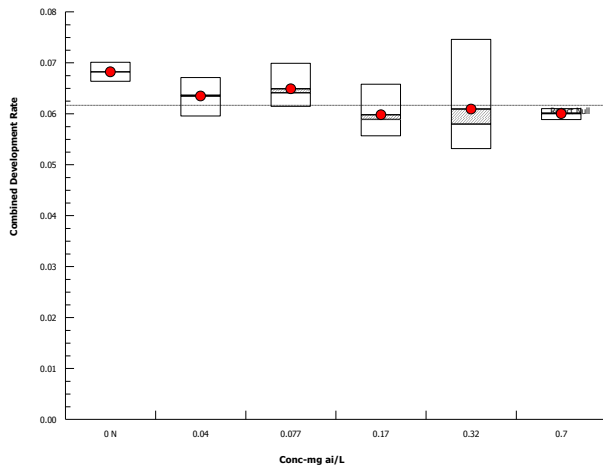
### Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance Test	12.4	15.1	0.0293	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.912	0.884	0.0384	Normal Distribution

### Combined Development Rate Summary

Conc-mg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	0.0682	0.0648	0.0716	0.0682	0.0663	0.0701	0.00107	3.13%	0.00%
0.04		4	0.0635	0.0572	0.0697	0.0636	0.0595	0.0671	0.00195	6.16%	6.96%
0.077		4	0.0649	0.0584	0.0714	0.0641	0.0614	0.0699	0.00204	6.30%	4.88%
0.17		4	0.0598	0.0524	0.0671	0.0588	0.0556	0.0658	0.00231	7.72%	12.35%
0.32		4	0.0609	0.0459	0.0759	0.0579	0.0531	0.0746	0.00471	15.46%	10.70%
0.7		4	0.06	0.0585	0.0615	0.0601	0.0588	0.061	0.000481	1.60%	12.02%

### Graphics



# CETIS Analytical Report

Report Date: 30 Jun-17 18:45 (p 4 of 14)  
Test Code: 125618 49910308 | 18-9896-9629

## OPPTS 850.1760 Chronic Sediment Chironomus Huntingdon Life Sciences

<b>Analysis ID:</b> 07-4043-9874	<b>Endpoint:</b> Female Development Rate	<b>CETIS Version:</b> CETISv1.9.2
<b>Analyzed:</b> 30 Jun-17 18:41	<b>Analysis:</b> Parametric-Two Sample	<b>Official Results:</b> Yes
<b>Batch ID:</b> 08-0420-8106	<b>Test Type:</b> Chronic Sediment Chironomus	<b>Analyst:</b>
<b>Start Date:</b> 28 Oct-05	<b>Protocol:</b> OPPTS 850.1760 Chronic Sediment Chiron	<b>Diluent:</b> Elendt M4 Medium
<b>Ending Date:</b>	<b>Species:</b> Chironomus dilutus	<b>Brine:</b> Not Applicable
<b>Duration:</b> n/a	<b>Source:</b> Lab In-House Culture	<b>Age:</b> <36h

Data Transform	Alt Hyp	Comparison Result	PMSD
Untransformed	C <> T	Solvent Blank passed female development rat	8.31%

### Equal Variance t Two-Sample Test

Control	vs	Control II	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		Solvent Blank	0.607	2.23	0.005	10	CDF	0.5573	Non-Significant Effect

### ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	5.042E-06	5.042E-06	1	0.369	0.5573	Non-Significant Effect
Error	0.0001368	1.368E-05	10			
Total	0.0001418		11			

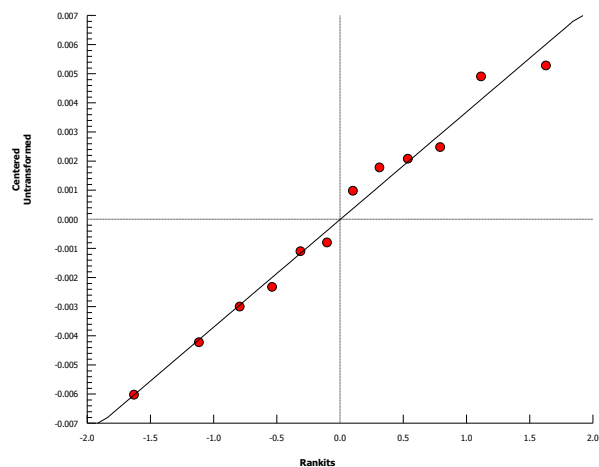
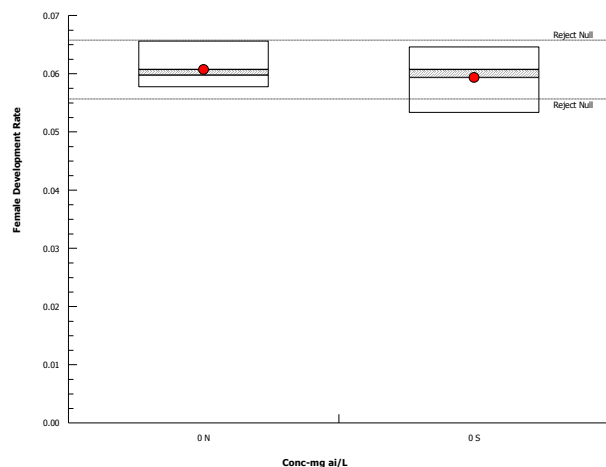
### Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F Test	1.25	44.4	0.9340	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.971	0.802	0.9196	Normal Distribution

### Female Development Rate Summary

Conc-mg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	S	8	0.0593	0.0561	0.0625	0.0607	0.0533	0.0646	0.00135	6.43%	0.00%
0	N	4	0.0607	0.0553	0.0661	0.0597	0.0577	0.0656	0.0017	5.62%	-2.32%

### Graphics



# CETIS Analytical Report

Report Date: 30 Jun-17 18:45 (p 5 of 14)  
Test Code: 125618 49910308 | 18-9896-9629

## OPPTS 850.1760 Chronic Sediment Chironomus Huntingdon Life Sciences

<b>Analysis ID:</b> 11-8638-5730	<b>Endpoint:</b> Female Development Rate	<b>CETIS Version:</b> CETISv1.9.2
<b>Analyzed:</b> 30 Jun-17 18:42	<b>Analysis:</b> Parametric-Control vs Treatments	<b>Official Results:</b> Yes
<b>Batch ID:</b> 08-0420-8106	<b>Test Type:</b> Chronic Sediment Chironomus	<b>Analyst:</b>
<b>Start Date:</b> 28 Oct-05	<b>Protocol:</b> OPPTS 850.1760 Chronic Sediment Chiron	<b>Diluent:</b> Elendt M4 Medium
<b>Ending Date:</b>	<b>Species:</b> Chironomus dilutus	<b>Brine:</b> Not Applicable
<b>Duration:</b> n/a	<b>Source:</b> Lab In-House Culture	<b>Age:</b> <36h

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	0.32	0.7	0.4733		15.06%

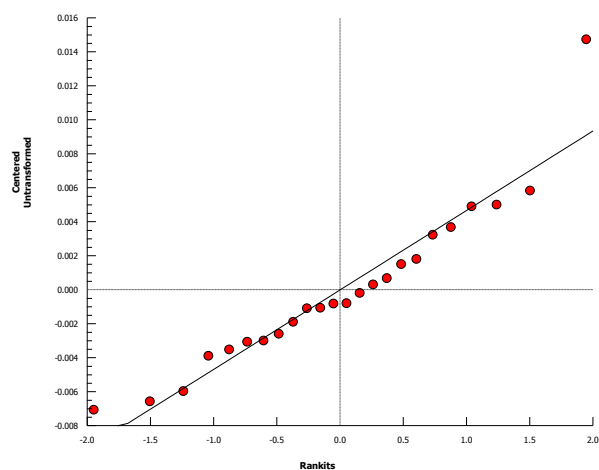
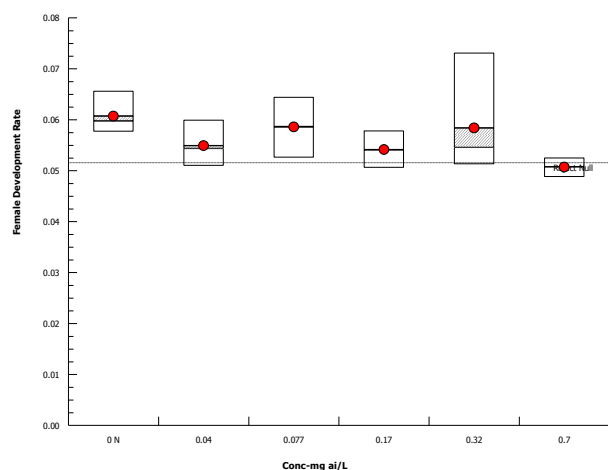
Dunnett Multiple Comparison Test									
Control	vs	Conc-mg ai/L	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		0.04	1.53	2.41	0.009	6	CDF	0.2192	Non-Significant Effect
		0.077	0.56	2.41	0.009	6	CDF	0.6213	Non-Significant Effect
		0.17	1.73	2.41	0.009	6	CDF	0.1616	Non-Significant Effect
		0.32	0.612	2.41	0.009	6	CDF	0.5977	Non-Significant Effect
		0.7*	2.63	2.41	0.009	6	CDF	0.0322	Significant Effect

ANOVA Table						
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0002674	5.349E-05	5	1.86	0.1526	Non-Significant Effect
Error	0.0005189	2.883E-05	18			
Total	0.0007863		23			

Distributional Tests						
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)	
Variances	Bartlett Equality of Variance Test	10.2	15.1	0.0701	Equal Variances	
Distribution	Shapiro-Wilk W Normality Test	0.923	0.884	0.0672	Normal Distribution	

Female Development Rate Summary											
Conc-mg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	0.0607	0.0553	0.0661	0.0597	0.0577	0.0656	0.0017	5.62%	0.00%
0.04		4	0.0549	0.0485	0.0613	0.0543	0.051	0.0599	0.00203	7.38%	9.56%
0.077		4	0.0586	0.0499	0.0673	0.0587	0.0526	0.0644	0.00273	9.32%	3.50%
0.17		4	0.0541	0.0493	0.0589	0.054	0.0506	0.0578	0.0015	5.55%	10.83%
0.32		4	0.0584	0.0422	0.0746	0.0545	0.0513	0.0731	0.00509	17.45%	3.83%
0.7		4	0.0507	0.0483	0.0531	0.0508	0.0488	0.0525	0.000763	3.01%	16.47%

### Graphics





# CETIS Analytical Report

Report Date: 30 Jun-17 18:45 (p 6 of 14)  
Test Code: 125618 49910308 | 18-9896-9629

## OPPTS 850.1760 Chronic Sediment Chironomus

Huntingdon Life Sciences

<b>Analysis ID:</b> 04-4457-6781	<b>Endpoint:</b> Female Development Rate	<b>CETIS Version:</b> CETISv1.9.2
<b>Analyzed:</b> 30 Jun-17 18:43	<b>Analysis:</b> Parametric-Control vs Ord.Treatments	<b>Official Results:</b> Yes
<b>Batch ID:</b> 08-0420-8106	<b>Test Type:</b> Chronic Sediment Chironomus	<b>Analyst:</b>
<b>Start Date:</b> 28 Oct-05	<b>Protocol:</b> OPPTS 850.1760 Chronic Sediment Chiron	<b>Diluent:</b> Elendt M4 Medium
<b>Ending Date:</b>	<b>Species:</b> Chironomus dilutus	<b>Brine:</b> Not Applicable
<b>Duration:</b> n/a	<b>Source:</b> Lab In-House Culture	<b>Age:</b> <36h

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	0.32	0.7	0.4733		11.68%

### Williams Multiple Comparison Test

Control	vs	Conc-mg ai/L	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		0.04	1.53	1.73	0.007	6	CDF	>0.05	Non-Significant Effect
		0.077	1.04	1.82	0.007	6	CDF	>0.05	Non-Significant Effect
		0.17	1.73	1.85	0.007	6	CDF	>0.05	Non-Significant Effect
		0.32	1.17	1.86	0.007	6	CDF	>0.05	Non-Significant Effect
		0.7*	2.63	1.87	0.007	6	CDF	<0.05	Significant Effect

### ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0002674	5.349E-05	5	1.86	0.1526	Non-Significant Effect
Error	0.0005189	2.883E-05	18			
Total	0.0007863		23			

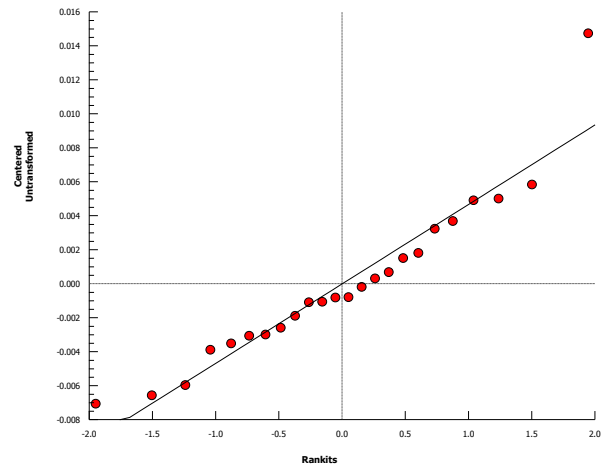
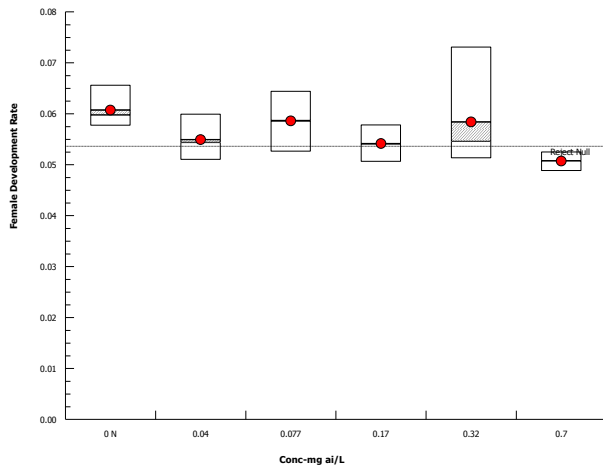
### Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance Test	10.2	15.1	0.0701	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.923	0.884	0.0672	Normal Distribution

### Female Development Rate Summary

Conc-mg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	0.0607	0.0553	0.0661	0.0597	0.0577	0.0656	0.0017	5.62%	0.00%
0.04		4	0.0549	0.0485	0.0613	0.0543	0.051	0.0599	0.00203	7.38%	9.56%
0.077		4	0.0586	0.0499	0.0673	0.0587	0.0526	0.0644	0.00273	9.32%	3.50%
0.17		4	0.0541	0.0493	0.0589	0.054	0.0506	0.0578	0.0015	5.55%	10.83%
0.32		4	0.0584	0.0422	0.0746	0.0545	0.0513	0.0731	0.00509	17.45%	3.83%
0.7		4	0.0507	0.0483	0.0531	0.0508	0.0488	0.0525	0.000763	3.01%	16.47%

### Graphics



# CETIS Analytical Report

Report Date: 30 Jun-17 18:45 (p 7 of 14)  
Test Code: 125618 49910308 | 18-9896-9629

## OPPTS 850.1760 Chronic Sediment Chironomus Huntingdon Life Sciences

<b>Analysis ID:</b> 07-9635-0595	<b>Endpoint:</b> Male Development Rate	<b>CETIS Version:</b> CETISv1.9.2
<b>Analyzed:</b> 30 Jun-17 18:41	<b>Analysis:</b> Parametric-Two Sample	<b>Official Results:</b> Yes
<b>Batch ID:</b> 08-0420-8106	<b>Test Type:</b> Chronic Sediment Chironomus	<b>Analyst:</b>
<b>Start Date:</b> 28 Oct-05	<b>Protocol:</b> OPPTS 850.1760 Chronic Sediment Chiron	<b>Diluent:</b> Elendt M4 Medium
<b>Ending Date:</b>	<b>Species:</b> Chironomus dilutus	<b>Brine:</b> Not Applicable
<b>Duration:</b> n/a	<b>Source:</b> Lab In-House Culture	<b>Age:</b> <36h

Data Transform	Alt Hyp	Comparison Result	PMSD
Untransformed	C <> T	Solvent Blank failed male development rate	4.30%

### Equal Variance t Two-Sample Test

Control	vs	Control II	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		Solvent Blank*	2.5	2.23	0.003	10	CDF	0.0312	Significant Effect

### ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	3.602E-05	3.602E-05	1	6.27	0.0312	Significant Effect
Error	5.743E-05	5.743E-06	10			
Total	9.344E-05		11			

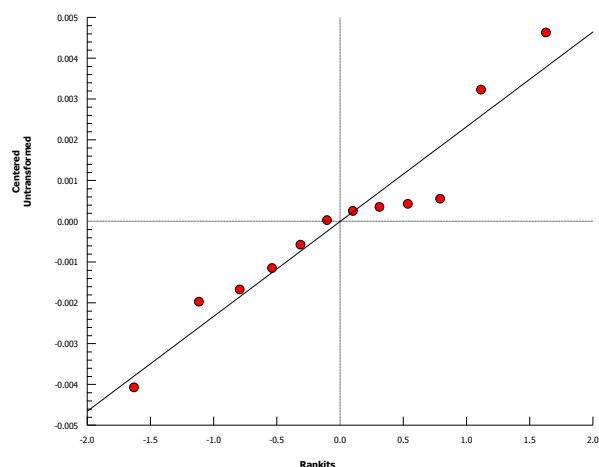
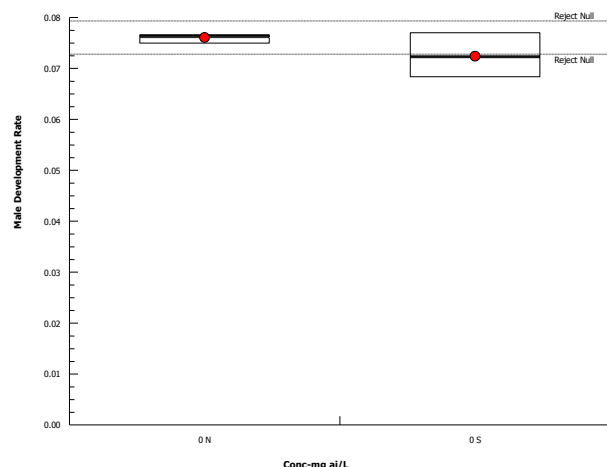
### Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F Test	13.2	44.4	0.0580	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.942	0.802	0.5298	Normal Distribution

### Male Development Rate Summary

Conc-mg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	S	8	0.0724	0.07	0.0747	0.0721	0.0683	0.077	0.000997	3.89%	0.00%
0	N	4	0.0761	0.0748	0.0773	0.0764	0.0749	0.0766	0.000388	1.02%	-5.08%

### Graphics



# CETIS Analytical Report

Report Date: 30 Jun-17 18:45 (p 8 of 14)  
Test Code: 125618 49910308 | 18-9896-9629

## OPPTS 850.1760 Chronic Sediment Chironomus

Huntingdon Life Sciences

<b>Analysis ID:</b> 13-1879-6199	<b>Endpoint:</b> Male Development Rate	<b>CETIS Version:</b> CETISv1.9.2
<b>Analyzed:</b> 30 Jun-17 18:42	<b>Analysis:</b> Parametric-Control vs Treatments	<b>Official Results:</b> Yes
<b>Batch ID:</b> 08-0420-8106	<b>Test Type:</b> Chronic Sediment Chironomus	<b>Analyst:</b>
<b>Start Date:</b> 28 Oct-05	<b>Protocol:</b> OPPTS 850.1760 Chronic Sediment Chiron	<b>Diluent:</b> Elendt M4 Medium
<b>Ending Date:</b>	<b>Species:</b> Chironomus dilutus	<b>Brine:</b> Not Applicable
<b>Duration:</b> n/a	<b>Source:</b> Lab In-House Culture	<b>Age:</b> <36h

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	0.17	0.32	0.2332		10.78%

### Dunnett Multiple Comparison Test

Control	vs	Conc-mg ai/L	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		0.04	1.81	2.41	0.008	6	CDF	0.1422	Non-Significant Effect
		0.077	0.558	2.41	0.008	6	CDF	0.6222	Non-Significant Effect
		0.17	2.14	2.41	0.008	6	CDF	0.0823	Non-Significant Effect
		0.32*	3.21	2.41	0.008	6	CDF	0.0098	Significant Effect
		0.7	2.4	2.41	0.008	6	CDF	0.0507	Non-Significant Effect

### ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0003332	6.665E-05	5	2.87	0.0445	Significant Effect
Error	0.0004177	2.321E-05	18			
Total	0.000751		23			

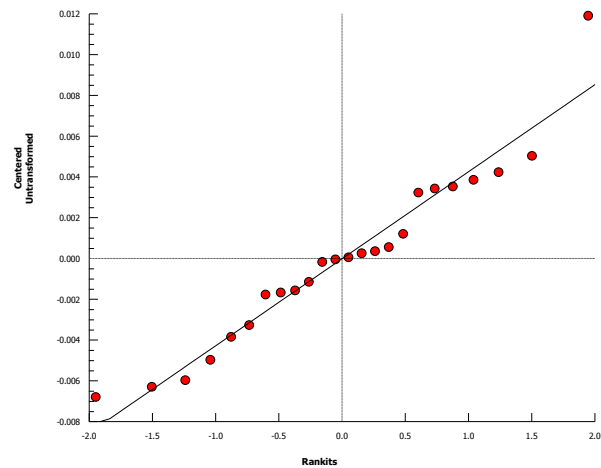
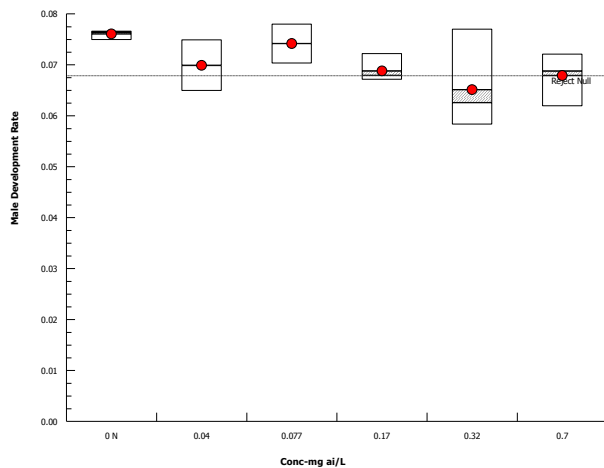
### Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance Test	12.5	15.1	0.0286	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.95	0.884	0.2662	Normal Distribution

### Male Development Rate Summary

Conc-mg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	0.0761	0.0748	0.0773	0.0764	0.0749	0.0766	0.000388	1.02%	0.00%
0.04		4	0.0699	0.0621	0.0776	0.0698	0.0649	0.0749	0.00243	6.97%	8.12%
0.077		4	0.0741	0.0691	0.0792	0.0741	0.0703	0.078	0.00157	4.24%	2.50%
0.17		4	0.0688	0.065	0.0726	0.0679	0.0671	0.0722	0.00119	3.47%	9.57%
0.32		4	0.0651	0.0512	0.079	0.0626	0.0583	0.077	0.00437	13.42%	14.40%
0.7		4	0.0679	0.0602	0.0755	0.0688	0.0619	0.0721	0.0024	7.07%	10.75%

### Graphics



# CETIS Analytical Report

Report Date: 30 Jun-17 18:45 (p 9 of 14)  
Test Code: 125618 49910308 | 18-9896-9629

## OPPTS 850.1760 Chronic Sediment Chironomus

Huntingdon Life Sciences

<b>Analysis ID:</b> 06-3049-0380	<b>Endpoint:</b> Male Development Rate	<b>CETIS Version:</b> CETISv1.9.2
<b>Analyzed:</b> 30 Jun-17 18:43	<b>Analysis:</b> Parametric-Control vs Ord.Treatments	<b>Official Results:</b> Yes
<b>Batch ID:</b> 08-0420-8106	<b>Test Type:</b> Chronic Sediment Chironomus	<b>Analyst:</b>
<b>Start Date:</b> 28 Oct-05	<b>Protocol:</b> OPPTS 850.1760 Chronic Sediment Chiron	<b>Diluent:</b> Elendt M4 Medium
<b>Ending Date:</b>	<b>Species:</b> Chironomus dilutus	<b>Brine:</b> Not Applicable
<b>Duration:</b> n/a	<b>Source:</b> Lab In-House Culture	<b>Age:</b> <36h

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	0.077	0.17	0.1144		8.36%

### Williams Multiple Comparison Test

Control	vs	Conc-mg ai/L	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		0.04	1.81	1.73	0.006	6	CDF	>0.05	Non-Significant Effect
		0.077	1.19	1.82	0.006	6	CDF	>0.05	Non-Significant Effect
		0.17*	2.14	1.85	0.006	6	CDF	<0.05	Significant Effect
		0.32*	3.21	1.86	0.006	6	CDF	<0.05	Significant Effect
		0.7*	2.81	1.87	0.006	6	CDF	<0.05	Significant Effect

### ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0003332	6.665E-05	5	2.87	0.0445	Significant Effect
Error	0.0004177	2.321E-05	18			
Total	0.000751		23			

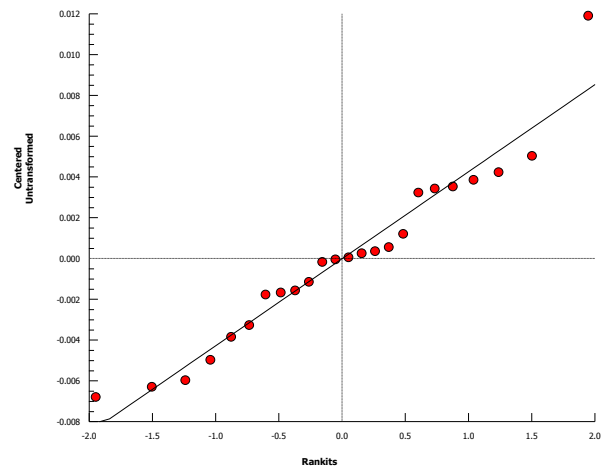
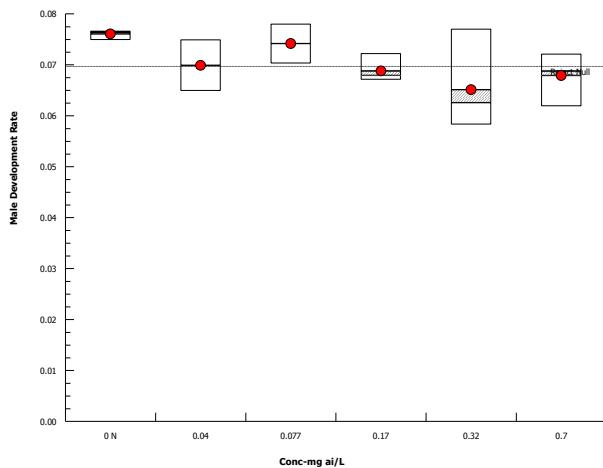
### Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance Test	12.5	15.1	0.0286	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.95	0.884	0.2662	Normal Distribution

### Male Development Rate Summary

Conc-mg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	0.0761	0.0748	0.0773	0.0764	0.0749	0.0766	0.000388	1.02%	0.00%
0.04		4	0.0699	0.0621	0.0776	0.0698	0.0649	0.0749	0.00243	6.97%	8.12%
0.077		4	0.0741	0.0691	0.0792	0.0741	0.0703	0.078	0.00157	4.24%	2.50%
0.17		4	0.0688	0.065	0.0726	0.0679	0.0671	0.0722	0.00119	3.47%	9.57%
0.32		4	0.0651	0.0512	0.079	0.0626	0.0583	0.077	0.00437	13.42%	14.40%
0.7		4	0.0679	0.0602	0.0755	0.0688	0.0619	0.0721	0.0024	7.07%	10.75%

### Graphics



# CETIS Analytical Report

Report Date: 30 Jun-17 18:45 (p 10 of 14)  
Test Code: 125618 49910308 | 18-9896-9629

## OPPTS 850.1760 Chronic Sediment Chironomus Huntingdon Life Sciences

<b>Analysis ID:</b> 02-9674-1904	<b>Endpoint:</b> Male to Female Ratio	<b>CETIS Version:</b> CETISv1.9.2
<b>Analyzed:</b> 30 Jun-17 18:41	<b>Analysis:</b> Parametric-Two Sample	<b>Official Results:</b> Yes
<b>Batch ID:</b> 08-0420-8106	<b>Test Type:</b> Chronic Sediment Chironomus	<b>Analyst:</b>
<b>Start Date:</b> 28 Oct-05	<b>Protocol:</b> OPPTS 850.1760 Chronic Sediment Chiron	<b>Diluent:</b> Elendt M4 Medium
<b>Ending Date:</b>	<b>Species:</b> Chironomus dilutus	<b>Brine:</b> Not Applicable
<b>Duration:</b> n/a	<b>Source:</b> Lab In-House Culture	<b>Age:</b> <36h

Data Transform	Alt Hyp	Comparison Result	PMSD
Untransformed	C <> T	Solvent Blank passed male to female ratio	53.22%

### Equal Variance t Two-Sample Test

Control	vs	Control II	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		Solvent Blank	0.384	2.23	0.559	10	CDF	0.7092	Non-Significant Effect

### ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0247042	0.0247042	1	0.147	0.7092	Non-Significant Effect
Error	1.67739	0.167739	10			
Total	1.70209		11			

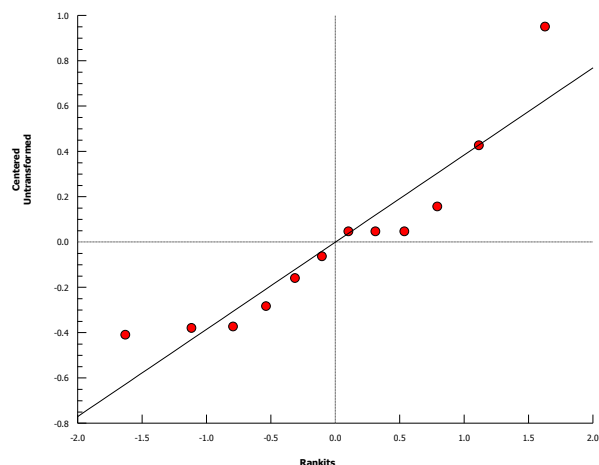
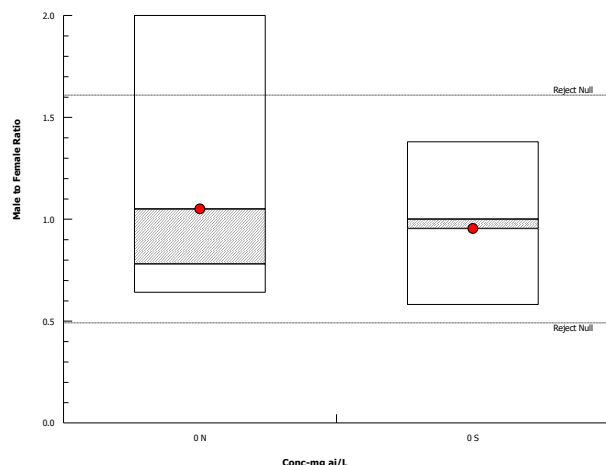
### Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F Test	6.63	10.9	0.0375	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.875	0.802	0.0766	Normal Distribution

### Male to Female Ratio Summary

Conc-mg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	S	8	0.954	0.745	1.16	1	0.58	1.38	0.0883	26.19%	0.00%
0	N	4	1.05	0.0267	2.07	0.78	0.64	2	0.322	61.24%	-10.09%

### Graphics



# CETIS Analytical Report

Report Date: 30 Jun-17 18:45 (p 11 of 14)  
Test Code: 125618 49910308 | 18-9896-9629

## OPPTS 850.1760 Chronic Sediment Chironomus

Huntingdon Life Sciences

Analysis ID: 18-2194-7084	Endpoint: Male to Female Ratio	CETIS Version: CETISv1.9.2
Analyzed: 30 Jun-17 18:42	Analysis: Nonparametric-Two Sample	Official Results: Yes
Batch ID: 08-0420-8106	Test Type: Chronic Sediment Chironomus	Analyst:
Start Date: 28 Oct-05	Protocol: OPPTS 850.1760 Chronic Sediment Chiron	Diluent: Elendt M4 Medium
Ending Date:	Species: Chironomus dilutus	Brine: Not Applicable
Duration: n/a	Source: Lab In-House Culture	Age: <36h

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	0.7	> 0.7	n/a		354.49%

### Mann-Whitney U Two-Sample Test

Control	vs	Conc-mg ai/L	Test Stat	Critical	Ties	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		0.04	4	n/a	0	6	Exact	0.9000	Non-Significant Effect
		0.077	10	n/a	0	6	Exact	0.3143	Non-Significant Effect
		0.17	11	n/a	0	6	Exact	0.2429	Non-Significant Effect
		0.32	12.5	n/a	1	6	Exact	0.1143	Non-Significant Effect
		0.7	7	n/a	0	6	Exact	0.6571	Non-Significant Effect

### ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	37.2652	7.45303	5	1.02	0.4373	Non-Significant Effect
Error	132.091	7.3384	18			
Total	169.356		23			

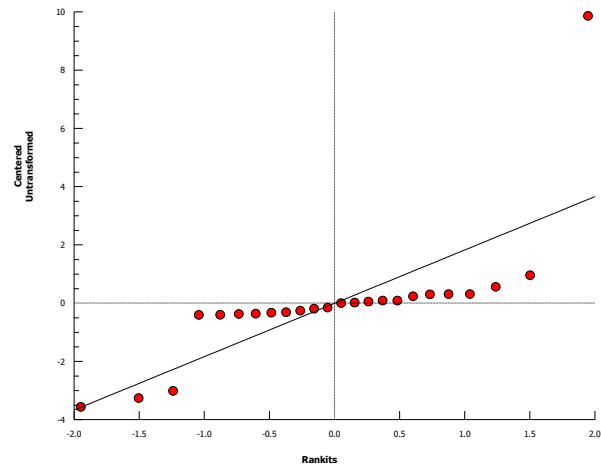
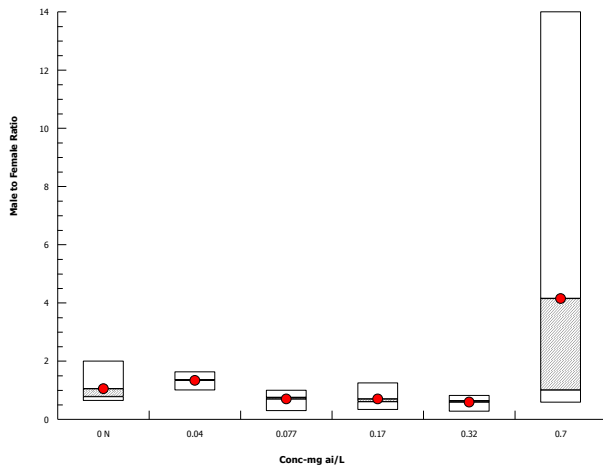
### Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance Test	49.1	15.1	<1.0E-37	Unequal Variances
Distribution	Shapiro-Wilk W Normality Test	0.585	0.884	4.2E-07	Non-Normal Distribution

### Male to Female Ratio Summary

Conc-mg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	1.05	0.0267	2.07	0.78	0.64	2	0.322	61.24%	0.00%
0.04		4	1.34	0.923	1.75	1.36	1	1.63	0.13	19.40%	-27.14%
0.077		4	0.697	0.125	1.27	0.75	0.29	1	0.18	51.56%	33.57%
0.17		4	0.697	0.0359	1.36	0.605	0.33	1.25	0.208	59.61%	33.57%
0.32		4	0.59	0.22	0.96	0.635	0.27	0.82	0.116	39.36%	43.81%
0.7		4	4.15	-6.31	14.6	1	0.58	14	3.29	158.46%	-295.00%

### Graphics



# CETIS Analytical Report

Report Date: 30 Jun-17 18:45 (p 12 of 14)  
Test Code: 125618 49910308 | 18-9896-9629

## OPPTS 850.1760 Chronic Sediment Chironomus Huntingdon Life Sciences

<b>Analysis ID:</b> 16-0059-1876	<b>Endpoint:</b> Percent Emerged	<b>CETIS Version:</b> CETISv1.9.2
<b>Analyzed:</b> 30 Jun-17 18:41	<b>Analysis:</b> Parametric-Two Sample	<b>Official Results:</b> Yes
<b>Batch ID:</b> 08-0420-8106	<b>Test Type:</b> Chronic Sediment Chironomus	<b>Analyst:</b>
<b>Start Date:</b> 28 Oct-05	<b>Protocol:</b> OPPTS 850.1760 Chronic Sediment Chiron	<b>Diluent:</b> Elendt M4 Medium
<b>Ending Date:</b>	<b>Species:</b> Chironomus dilutus	<b>Brine:</b> Not Applicable
<b>Duration:</b> n/a	<b>Source:</b> Lab In-House Culture	<b>Age:</b> <36h

Data Transform	Alt Hyp	Comparison Result	PMSD
Untransformed	C <> T	Solvent Blank passed percent emerged	8.27%

### Equal Variance t Two-Sample Test

Control	vs	Control II	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		Solvent Blank	0.738	2.23	0.076	10	CDF	0.4776	Non-Significant Effect

### ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0016667	0.0016667	1	0.544	0.4776	Non-Significant Effect
Error	0.030625	0.0030625	10			
Total	0.0322917		11			

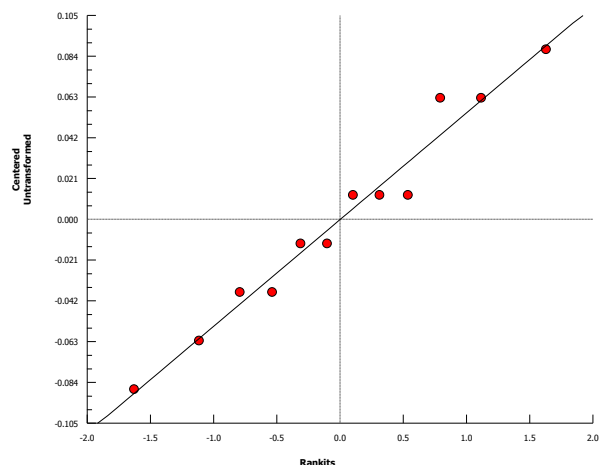
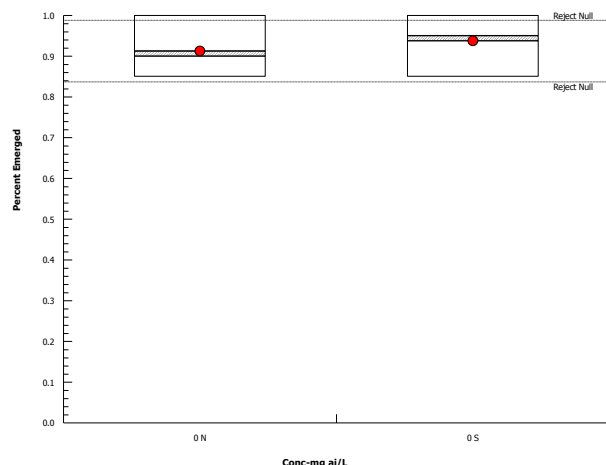
### Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F Test	1.48	10.9	0.6021	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.964	0.802	0.8418	Normal Distribution

### Percent Emerged Summary

Conc-mg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	S	8	0.937	0.894	0.981	0.950	0.850	1.000	0.018	5.52%	0.00%
0	N	4	0.912	0.812	1.000	0.900	0.850	1.000	0.032	6.89%	2.67%

### Graphics



# CETIS Analytical Report

Report Date: 30 Jun-17 18:45 (p 13 of 14)  
Test Code: 125618 49910308 | 18-9896-9629

## OPPTS 850.1760 Chronic Sediment Chironomus

Huntingdon Life Sciences

<b>Analysis ID:</b> 07-6919-2596	<b>Endpoint:</b> Percent Emerged	<b>CETIS Version:</b> CETISv1.9.2
<b>Analyzed:</b> 30 Jun-17 18:42	<b>Analysis:</b> Parametric-Control vs Treatments	<b>Official Results:</b> Yes
<b>Batch ID:</b> 08-0420-8106	<b>Test Type:</b> Chronic Sediment Chironomus	<b>Analyst:</b>
<b>Start Date:</b> 28 Oct-05	<b>Protocol:</b> OPPTS 850.1760 Chronic Sediment Chiron	<b>Diluent:</b> Elendt M4 Medium
<b>Ending Date:</b>	<b>Species:</b> Chironomus dilutus	<b>Brine:</b> Not Applicable
<b>Duration:</b> n/a	<b>Source:</b> Lab In-House Culture	<b>Age:</b> <36h

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	0.7	> 0.7	n/a		18.06%

### Dunnett Multiple Comparison Test

Control	vs	Conc-mg ai/L	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		0.04	0.365	2.41	0.165	6	CDF	0.7043	Non-Significant Effect
		0.077	-0.183	2.41	0.165	6	CDF	0.8814	Non-Significant Effect
		0.17	0.548	2.41	0.165	6	CDF	0.6266	Non-Significant Effect
		0.32	0.548	2.41	0.165	6	CDF	0.6266	Non-Significant Effect
		0.7	1.28	2.41	0.165	6	CDF	0.3064	Non-Significant Effect

### ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0245833	0.0049167	5	0.524	0.7547	Non-Significant Effect
Error	0.16875	0.009375	18			
Total	0.193333		23			

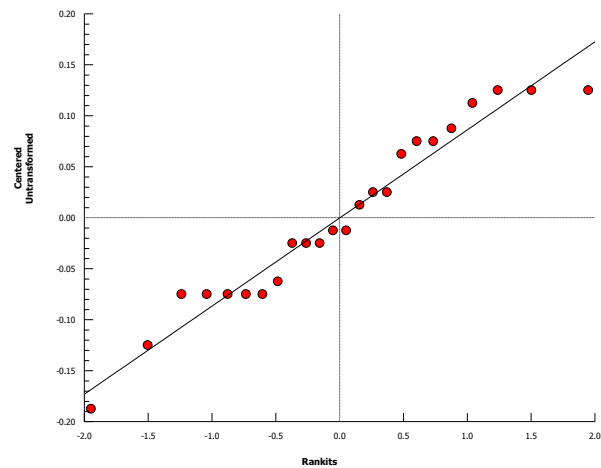
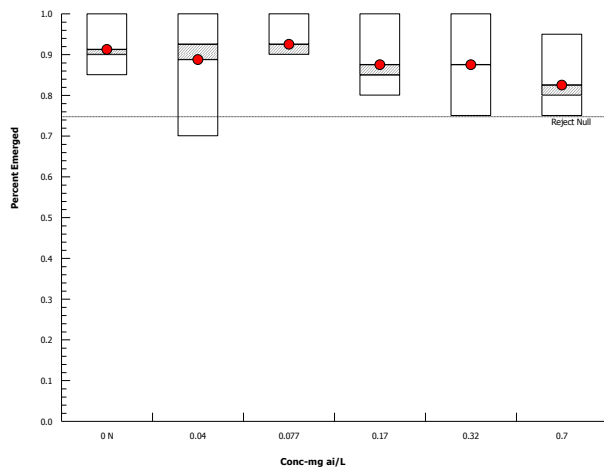
### Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance Test	3.2	15.1	0.6696	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.949	0.884	0.2568	Normal Distribution

### Percent Emerged Summary

Conc-mg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	0.912	0.812	1.000	0.900	0.850	1.000	0.032	6.89%	0.00%
0.04		4	0.887	0.678	1.000	0.925	0.700	1.000	0.066	14.82%	2.74%
0.077		4	0.925	0.845	1.000	0.900	0.900	1.000	0.025	5.41%	-1.37%
0.17		4	0.875	0.723	1.000	0.850	0.800	1.000	0.048	10.94%	4.11%
0.32		4	0.875	0.686	1.000	0.875	0.750	1.000	0.060	13.60%	4.11%
0.7		4	0.825	0.673	0.977	0.800	0.750	0.950	0.048	11.61%	9.59%

### Graphics





# CETIS Analytical Report

Report Date: 30 Jun-17 18:45 (p 14 of 14)  
Test Code: 125618 49910308 | 18-9896-9629

OPPTS 850.1760 Chronic Sediment Chironomus Huntingdon Life Sciences

<b>Analysis ID:</b> 00-1511-9652	<b>Endpoint:</b> Percent Emerged	<b>CETIS Version:</b> CETISv1.9.2
<b>Analyzed:</b> 30 Jun-17 18:43	<b>Analysis:</b> Parametric-Control vs Ord.Treatments	<b>Official Results:</b> Yes
<b>Batch ID:</b> 08-0420-8106	<b>Test Type:</b> Chronic Sediment Chironomus	<b>Analyst:</b>
<b>Start Date:</b> 28 Oct-05	<b>Protocol:</b> OPPTS 850.1760 Chronic Sediment Chiron	<b>Diluent:</b> Elendt M4 Medium
<b>Ending Date:</b>	<b>Species:</b> Chironomus dilutus	<b>Brine:</b> Not Applicable
<b>Duration:</b> n/a	<b>Source:</b> Lab In-House Culture	<b>Age:</b> <36h

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	0.7	> 0.7	n/a		14.01%

## Williams Multiple Comparison Test

Control	vs	Conc-mg ai/L	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		0.04	0.365	1.73	0.119	6	CDF	>0.05	Non-Significant Effect
		0.077	0.0913	1.82	0.124	6	CDF	>0.05	Non-Significant Effect
		0.17	0.548	1.85	0.126	6	CDF	>0.05	Non-Significant Effect
		0.32	0.548	1.86	0.127	6	CDF	>0.05	Non-Significant Effect
		0.7	1.28	1.87	0.128	6	CDF	>0.05	Non-Significant Effect

## ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0245833	0.0049167	5	0.524	0.7547	Non-Significant Effect
Error	0.16875	0.009375	18			
Total	0.193333		23			

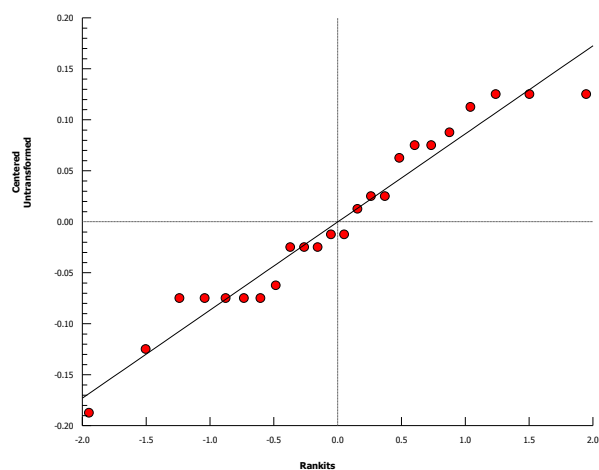
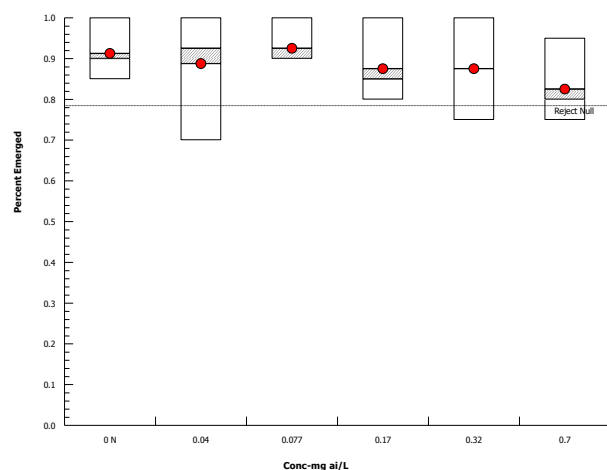
## Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance Test	3.2	15.1	0.6696	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.949	0.884	0.2568	Normal Distribution

## Percent Emerged Summary

Conc-mg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	0.912	0.812	1.000	0.900	0.850	1.000	0.032	6.89%	0.00%
0.04		4	0.887	0.678	1.000	0.925	0.700	1.000	0.066	14.82%	2.74%
0.077		4	0.925	0.845	1.000	0.900	0.900	1.000	0.025	5.41%	-1.37%
0.17		4	0.875	0.723	1.000	0.850	0.800	1.000	0.048	10.94%	4.11%
0.32		4	0.875	0.686	1.000	0.875	0.750	1.000	0.060	13.60%	4.11%
0.7		4	0.825	0.673	0.977	0.800	0.750	0.950	0.048	11.61%	9.59%

## Graphics



# CETIS Summary Report

Report Date: 30 Jun-17 18:45 (p 1 of 3)  
Test Code: 125618 49910308 | 18-9896-9629

## OPPTS 850.1760 Chronic Sediment Chironomus

Huntingdon Life Sciences

Batch ID:	08-0420-8106	Test Type:	Chronic Sediment Chironomus	Analyst:	
Start Date:	28 Oct-05	Protocol:	OPPTS 850.1760 Chronic Sediment Chiron	Diluent:	Elendt M4 Medium
Ending Date:		Species:	Chironomus dilutus	Brine:	Not Applicable
Duration:	n/a	Source:	Lab In-House Culture	Age:	<36h
Sample ID:	19-2825-0411	Code:	125618 49910308	Client:	CDM Smith - J. Marton
Sample Date:	28 Oct-05	Material:	Ipconazole	Project:	Fungicide
Receipt Date:		Source:	Kureha Corporation		
Sample Age:	n/a	Station:			

### Comments:

PC Code 125618, MRID 49910308, TWA overlying water concentrations

'Combined Development Rate' endpoint...

As configured, the Williams Multiple Comparison Test assumes a monotonically decreasing concentration-response trend, however, the data set is not monotonically decreasing...CETIS will continue the calculation by smoothing the data.

-----  
'Female Development Rate' endpoint...

As configured, the Williams Multiple Comparison Test assumes a monotonically decreasing concentration-response trend, however, the data set is not monotonically decreasing...CETIS will continue the calculation by smoothing the data.

-----  
'Male Development Rate' endpoint...

As configured, the Williams Multiple Comparison Test assumes a monotonically decreasing concentration-response trend, however, the data set is not monotonically decreasing...CETIS will continue the calculation by smoothing the data.

-----  
'Percent Emerged' endpoint...

As configured, the Williams Multiple Comparison Test assumes a monotonically decreasing concentration-response trend, however, the data set is not monotonically decreasing...CETIS will continue the calculation by smoothing the data.

PC Code 125618, MRID 49910308, TWA overlying water concentrations

### Single Comparison Summary

Analysis ID	Endpoint	Comparison Method	P-Value	Comparison Result
20-4171-5538	Combined Development Rat	Equal Variance t Two-Sample Test	0.1654	Solvent Blank passed combined developmen
07-4043-9874	Female Development Rate	Equal Variance t Two-Sample Test	0.5573	Solvent Blank passed female development ra
07-9635-0595	Male Development Rate	Equal Variance t Two-Sample Test	0.0312	Solvent Blank failed male development rate
02-9674-1904	Male to Female Ratio	Equal Variance t Two-Sample Test	0.7092	Solvent Blank passed male to female ratio
16-0059-1876	Percent Emerged	Equal Variance t Two-Sample Test	0.4776	Solvent Blank passed percent emerged

### Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	NOEL	LOEL	TOEL	TU	PMSD ✓
03-0201-9941	Combined Development Rat	Dunnett Multiple Comparison Test	0.7	> 0.7	n/a		12.4%
18-5945-2666	Combined Development Rat	Williams Multiple Comparison Test	0.077	0.17	0.1144		9.59%
11-8638-5730	Female Development Rate	Dunnett Multiple Comparison Test	0.32	0.7	0.4733		15.1%
04-4457-6781	Female Development Rate	Williams Multiple Comparison Test	0.32	0.7	0.4733		11.7%
13-1879-6199	Male Development Rate	Dunnett Multiple Comparison Test	0.17	0.32	0.2332		10.8%
06-3049-0380	Male Development Rate	Williams Multiple Comparison Test	0.077	0.17	0.1144		8.36%
18-2194-7084	Male to Female Ratio	Mann-Whitney U Two-Sample Test	0.7	> 0.7	n/a		354.0
07-6919-2596	Percent Emerged	Dunnett Multiple Comparison Test	0.7	> 0.7	n/a		18.1%
00-1511-9652	Percent Emerged	Williams Multiple Comparison Test	0.7	> 0.7	n/a		14.0%

# CETIS Summary Report

Report Date: 30 Jun-17 18:45 (p 2 of 3)  
 Test Code: 125618 49910308 | 18-9896-9629

OPPTS 850.1760 Chronic Sediment Chironomus Huntingdon Life Sciences

Combined Development Rate Summary											
Conc-mg ai/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	S	8	0.0655	0.0629	0.0682	0.0588	0.0687	0.00113	0.0032	4.88%	0.00%
0	N	4	0.0682	0.0648	0.0716	0.0663	0.0701	0.00107	0.00214	3.13%	-4.08%
0.04		4	0.0635	0.0572	0.0697	0.0595	0.0671	0.00195	0.00391	6.16%	3.17%
0.077		4	0.0649	0.0584	0.0714	0.0614	0.0699	0.00204	0.00408	6.30%	0.99%
0.17		4	0.0598	0.0524	0.0671	0.0556	0.0658	0.00231	0.00461	7.72%	8.78%
0.32		4	0.0609	0.0459	0.0759	0.0531	0.0746	0.00471	0.00942	15.46%	7.06%
0.7		4	0.06	0.0585	0.0615	0.0588	0.061	0.000481	0.000963	1.60%	8.43%

Female Development Rate Summary											
Conc-mg ai/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	S	8	0.0593	0.0561	0.0625	0.0533	0.0646	0.00135	0.00382	6.43%	0.00%
0	N	4	0.0607	0.0553	0.0661	0.0577	0.0656	0.0017	0.00341	5.62%	-2.32%
0.04		4	0.0549	0.0485	0.0613	0.051	0.0599	0.00203	0.00405	7.38%	7.46%
0.077		4	0.0586	0.0499	0.0673	0.0526	0.0644	0.00273	0.00546	9.32%	1.26%
0.17		4	0.0541	0.0493	0.0589	0.0506	0.0578	0.0015	0.003	5.55%	8.77%
0.32		4	0.0584	0.0422	0.0746	0.0513	0.0731	0.00509	0.0102	17.45%	1.60%
0.7		4	0.0507	0.0483	0.0531	0.0488	0.0525	0.000763	0.00153	3.01%	14.54%

Male Development Rate Summary											
Conc-mg ai/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	S	8	0.0724	0.07	0.0747	0.0683	0.077	0.000997	0.00282	3.89%	0.00%
0	N	4	0.0761	0.0748	0.0773	0.0749	0.0766	0.000388	0.000777	1.02%	-5.08%
0.04		4	0.0699	0.0621	0.0776	0.0649	0.0749	0.00243	0.00487	6.97%	3.45%
0.077		4	0.0742	0.0691	0.0792	0.0703	0.078	0.00157	0.00314	4.24%	-2.45%
0.17		4	0.0688	0.065	0.0726	0.0671	0.0722	0.00119	0.00238	3.47%	4.97%
0.32		4	0.0651	0.0512	0.079	0.0583	0.077	0.00437	0.00874	13.42%	10.05%
0.7		4	0.0679	0.0602	0.0755	0.0619	0.0721	0.0024	0.0048	7.07%	6.22%

Male to Female Ratio Summary											
Conc-mg ai/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	S	8	0.954	0.745	1.16	0.58	1.38	0.0883	0.25	26.19%	0.00%
0	N	4	1.05	0.0267	2.07	0.64	2	0.322	0.643	61.24%	-10.09%
0.04		4	1.34	0.923	1.75	1	1.63	0.13	0.259	19.40%	-39.97%
0.077		4	0.698	0.125	1.27	0.29	1	0.18	0.36	51.56%	26.87%
0.17		4	0.698	0.0359	1.36	0.33	1.25	0.208	0.416	59.61%	26.87%
0.32		4	0.59	0.22	0.96	0.27	0.82	0.116	0.232	39.36%	38.14%
0.7		4	4.15	-6.31	14.6	0.58	14	3.29	6.57	158.46%	-334.86%

Percent Emerged Summary											
Conc-mg ai/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	S	8	0.938	0.894	0.981	0.850	1.000	0.018	0.052	5.52%	0.00%
0	N	4	0.912	0.812	1.000	0.850	1.000	0.032	0.063	6.89%	2.67%
0.04		4	0.888	0.678	1.000	0.700	1.000	0.066	0.131	14.82%	5.33%
0.077		4	0.925	0.845	1.000	0.900	1.000	0.025	0.050	5.41%	1.33%
0.17		4	0.875	0.723	1.000	0.800	1.000	0.048	0.096	10.94%	6.67%
0.32		4	0.875	0.686	1.000	0.750	1.000	0.060	0.119	13.60%	6.67%
0.7		4	0.825	0.673	0.977	0.750	0.950	0.048	0.096	11.61%	12.00%

# CETIS Summary Report

Report Date: 30 Jun-17 18:45 (p 3 of 3)  
Test Code: 125618 49910308 | 18-9896-9629

OPPTS 850.1760 Chronic Sediment Chironomus

Huntingdon Life Sciences

## Combined Development Rate Detail

Conc-mg ai/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	S	0.0639	0.0665	0.0664	0.0678	0.0588	0.0643	0.0687	0.0678
0	N	0.0701	0.07	0.0663	0.0664				
0.04		0.0671	0.0607	0.0665	0.0595				
0.077		0.0614	0.0665	0.0617	0.0699				
0.17		0.0556	0.0658	0.0609	0.0568				
0.32		0.058	0.0746	0.0531	0.0579				
0.7		0.0605	0.0597	0.0588	0.061				

## Female Development Rate Detail

Conc-mg ai/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	S	0.0551	0.0611	0.057	0.0646	0.0533	0.0603	0.0618	0.0614
0	N	0.0577	0.0656	0.0596	0.0599				
0.04		0.0564	0.051	0.0599	0.0523				
0.077		0.0526	0.0644	0.0555	0.0618				
0.17		0.0506	0.0578	0.0548	0.0533				
0.32		0.0513	0.0731	0.0518	0.0573				
0.7		0.051	0.0525	0.0505	0.0488				

## Male Development Rate Detail

Conc-mg ai/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	S	0.0728	0.0718	0.077	0.0707	0.0683	0.0704	0.0756	0.0724
0	N	0.0763	0.0749	0.0764	0.0766				
0.04		0.0749	0.0666	0.0731	0.0649				
0.077		0.0703	0.0741	0.0742	0.078				
0.17		0.0672	0.0722	0.0686	0.0671				
0.32		0.0663	0.077	0.0583	0.0588				
0.7		0.0714	0.0721	0.0661	0.0619				

## Male to Female Ratio Detail

Conc-mg ai/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	S	1	1	0.89	1.11	0.58	0.67	1	1.38
0	N	2	0.89	0.67	0.64				
0.04		1.38	1.63	1	1.33				
0.077		1	0.29	0.5	1				
0.17		0.43	1.25	0.78	0.33				
0.32		0.82	0.6	0.27	0.67				
0.7		0.88	0.58	1.13	14				

## Percent Emerged Detail

Conc-mg ai/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	S	1.000	0.900	0.850	0.950	0.950	1.000	0.900	0.950
0	N	0.900	0.850	1.000	0.900				
0.04		0.950	1.000	0.900	0.700				
0.077		1.000	0.900	0.900	0.900				
0.17		1.000	0.900	0.800	0.800				
0.32		1.000	0.800	0.950	0.750				
0.7		0.750	0.950	0.850	0.750				